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Description of a new loach species, *Yunnanilus triangulus* sp. nov. (Cypriniformes, Nemacheilidae), from Yunnan, China

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Abstract

A new species of the genus *Yunnanilus*, designated *Yunnanilus triangulus* **sp. nov.**, is described from the Nanpan River in Yunnan Province, southwestern China. Nuptial males of this species are distinguished by the presence of tubercles on the caudal peduncle during the reproductive period, a feature not observed in other members of the genus. The new species is further characterized by the following unique combination of morphological characters: body scaled, smaller eyes than interorbital width, nine inner gill rakers on first gill arch, lateral head length 27.1%–30.9% SL, head width 14.3%–17.2% SL, eye diameter 19.6%–23.8% of lateral head length, and caudal peduncle depth 94.0%–130.6% of its length. Morphological and molecular evidence support the validity of this species, despite its geographic proximity to *Y. polylepis*. These findings underscore the ecological specialization that exists within the genus *Yunnanilus* and highlight the urgent need for species-specific conservation strategies, given their restricted distributions and dependence on specific habitats.

Key Words

Caudal peduncle with tubercles, freshwater fish, morphological characters, sexual dimorphism

Introduction

Nichols (1925) designated *Nemacheilus pleurotaenia* Regan, 1904, as the type species of the nemacheilid loach subgenus *Yunnanilus*, with the following defining characters: short and compressed body, small scales, incomplete lateral line, slightly forked caudal fin, nostrils separated by greater distance than between posterior nostril and eye, and anterior nostril forming flap-like tube. Subsequently, Kottelat and Chu (1988) elevated *Yunnanilus* to the rank of genus, while Yang and Chen (1995) later categorized *Yunnanilus* species into two major assemblages, *Y. nigromaculatus* and *Y. pleurotaenia* species groups, based on the presence or absence of lateral and cephalic lateral line pores. Du et al. (2021) retained the *Y. pleurotaenia* group within *Yunnanilus* but reassigned the *Y. nigromaculatus* group to the revalidated genus *Eonemachilus* Berg, 1938. A more recent phylogenetic analysis of Chinese nemacheilids with tube-shaped anterior nostrils by Du et al. (2023) confirmed the generic status of *Yunnanilus* and refined its diagnostic characters to mouth inferior; anterior and posterior nostrils separated, anterior nostrils tube-like, without elongated barbel-like structure; cheeks scaleless; lips with furrows; and lateral line and cephalic lateral line pores present (Du et al. 2021, 2023).

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Currently, *Yunnanilus* comprises 20 valid species, all restricted to lakes, marshes, and slow-flowing waters within Yunnan and Sichuan Provinces of southwestern China (Qin et al. 2024). Among these, six species are distributed in the Yangtze River system, including *Y. discoloris* Zhou & He, 1989, *Y. longibulla* Yang, 1990, *Y. pleurotaenia* (Regan, 1904), and *Y. spanisbripes* An, Liu & Li, 2009 in the Jinsha River; *Y. sichuanensis* Ding, 1995 in the Yalong River; and *Y. jiuchiensis* Du, Hou, Chen & Yang, 2018 in the Tuo River; and 13 species are distributed in the Pearl River basin, including *Y. beipanjiangensis* Li, Mao & Sun, 1994 in the Beipan River; *Y. analis* Yang, 1990; *Y. chui* Yang, 1991; *Y. elakatis* Cao & Zhu, 1989; *Y. forkicaudalis* Li, 1999; *Y. macrogaster* Kottelat & Chu, 1988; *Y. macrolepis* Li, Tao & Mao, 2000; *Y. macrositanus* Li, 1999; *Y. nanpanjiangensis* Li, Tao & Lu, 1994; *Y. paludosus* Kottelat & Chu, 1988; *Y. parvus* Kottelat & Chu, 1988; *Y. polylepis* Qin, Shao, Du & Wang, 2024; and *Y. yangi* He et al., 2024 in the Nanpan River, and one species, *Y. chuanheensis* Jiang, Zhao, Du & Wang, 2021 in the Red River (Lixian River) (Du et al. 2021; Qin et al. 2024).

In April 2018, eight specimens of *Yunnanilus* were collected from a tributary of the Nanpan River in Huaning County, Yuxi City, Yunnan Province, China. Morphological and molecular analyses confirmed that these specimens represent a previously undescribed member of the genus, which is herein described and compared with all known congeners.

Materials and methods

All care and handling of experimental animals complied with the relevant laws of the Chinese Laboratory of Animal Welfare and Ethics (GB/T 35892-2018). Upon their collection, all specimens were rapidly euthanized by an overdose of anesthetic clove oil. The right-side pelvic fins of five individuals were removed and preserved in 99% ethanol for molecular analyses, and eight individuals were stored in 10% formalin for morphological comparisons. The specimens were deposited in the Kunming Natural History Museum of Zoology, Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS).

All counts and measurements followed the methodology described by Kottelat (1990). Initial data processing and preliminary statistical analyses were performed using Excel software. Genomic DNA was extracted from ethanol-preserved fin tissue, and partial sequences of the mitochondrial cytochrome c oxidase subunit I (COI) and cytochrome *b* (Cyt *b*) were sequenced by Tsingke Biotechnology Co., Ltd. (China). All sequences were assembled using SeqMan within the DNAStar package and aligned using MEGA v11.0 (Tamura et al. 2021). Sequences were submitted to GenBank (Accession Nos. PQ300642–PQ300646 for COI, PQ306053–PQ306057 for Cyt *b*). The phylogenetic position of *Yunnanilus triangulus* sp. nov. was determined using maximum-likelihood (ML) and Bayesian inference (BI) methods via the CIPRES Science Gateway (Miller et al. 2010). The ML tree was constructed using RAxML-HPC v8 (Stamatakis 2014) with a rapid bootstrapping configuration and 1000 bootstrap iterations. The BI tree was constructed using MrBayes in XSEDE v3.2.7a (Ronquist et al. 2012), with two simultaneous runs of four Markov chains starting from a random tree. The chains were run for five million generations and sampled every 100 generations. The first 25% of sampled trees were discarded as burn-in, and the remaining trees were used to generate a consensus tree and estimate Bayesian posterior probabilities (BPPs). The resulting phylogenetic trees were viewed and edited using Figtree v1.4.4.

Results

***Yunnanilus triangulus* sp. nov.**

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Holotype. • KIZ2018002410 female, 51.8 mm standard length (SL), Dalongtan Spring, Wangma Village, Huaning County, Yuxi City, Yunnan, P.R. China; Nanpan River, 24.1716°N, 102.9182°E, 1619 m a.s.l., X.Y. Chen, L.L. Xu, and H.D. Lyu, collected April 2018.

Paratypes. • Seven specimens. Female: KIZ2018002404, 2406–2407, 2409, 2411, 38.9–54.6 mm SL; Male: KIZ2018002405, KIZ2018002408, 37.1–39.1 mm SL; same as holotype.

Etymology. The specific name *triangulus* is derived from the unique presence of a tubercle on the caudal peduncle in nuptial males, a character not previously recorded in the genus *Yunnanilus*. We suggest the Chinese vernacular name "三角云南鳅" and the English vernacular name "triangle Yunnan loach." Gender: masculine.

Diagnosis. The new species can be distinguished from all other members of the genus by the presence of a unique triangular tubercle on the male caudal peduncle. It shares a body covered by scales, except on head and thorax, with *Y. chuanheensis*, *Y. jiuchiensis*, *Y. longibulla*, *Y. macrogaster*, *Y. macrolepis*, *Y. parvus*, *Y. pleurotaenia*, *Y. polylepis*, and *Y. spanisbripes*. However, the new species can be readily differentiated from these congeners by the following combination of characters: eye diameter shorter than interorbital width (vs. longer in *Y. jiuchiensis* and *Y. longibulla*), outer gill raker absent (vs. present in *Y. macrolepis* and *Y. spanisbripes*), processus dentiformis absent (vs. present in *Y. macrogaster*, *Y. parvus*, and *Y. pleurotaenia*), six branched pelvic-fin rays (vs. seven or eight in *Y. chuanheensis*), eight branched dorsal-fin rays (vs. nine in *Y. polylepis*), and specific metric characters.

Description. All morphometric and meristic data are given in Table 1. Greatest body depth anterior to dorsal fin origin, posterior portion gradually compressed from dorsal fin to caudal fin base. Lateral head longer than deep, deeper than wide. Snout slightly blunt, shorter than postorbital length of head. Eye diameter smaller than interorbital width; posterior nostril closer to anterior margin of eye than to tip of snout; anterior and posterior nostrils separated by distance greater than diameter of posterior nostril; base of anterior nostril tube-shaped, not elongated to barbel-like structure.

Table 1. Morphometric and meristic data of *Yunnanilus triangulus* sp. nov.

Characters	Holotype	Paratypes (mean ± SD)	
		Females (N = 5)	Males (N = 2)
Total length (mm)	51.8	49.6–67.3 (55.6 ± 7.0)	47.8–49.3 (48.6 ± 1.1)
Standard length (mm)	40.8	38.9–54.6 (44.1 ± 6.1)	37.1–39.1 (38.1 ± 1.4)
Percent of standard length (%)			
Deepest body depth	20.4	21.6–23.1 (22.1 ± 0.6)	20.9–21.2 (21.1 ± 0.2)
Head width	15.5	15.0–17.2 (15.7 ± 0.9)	14.3–14.4 (14.3 ± 0.0)
Lateral head length	27.7	27.1–29.0 (28.3 ± 0.8)	29.9–30.9 (30.4 ± 0.7)
Predorsal length	54.9	51.3–55.4 (53.8 ± 1.8)	53.6–54.8 (54.2 ± 0.8)
Prepelvic length	55.8	55.8–59.9 (58.1 ± 1.5)	56.9–57.7 (57.3 ± 0.5)
Preanal length	78.7	80.4–82.0 (81.4 ± 0.7)	80.2–81.7 (80.9 ± 1.1)
Preanus length	75.5	77.0–78.4 (77.5 ± 0.6)	77.1–77.2 (77.1 ± 0.1)
Caudal-peduncle length	13.0	9.4–12.1 (10.7 ± 1.0)	10.9–11.8 (11.3 ± 0.6)
Caudal-peduncle depth	12.2	11.5–12.8 (12.1 ± 0.5)	11.4–12.1 (11.8 ± 0.5)
Percent of Lateral head length (%)			
Head width	56.1	53.2–59.3 (55.5 ± 2.5)	46.2–48.0 (47.1 ± 1.2)
Head depth	60.0	55.1–60.9 (57.4 ± 2.2)	49.6–56.9 (53.2 ± 5.2)
Eye diameter	22.8	19.6–23.8 (21.0 ± 1.6)	21.3–21.4 (21.4 ± 0.0)
Interorbital width	23.9	21.3–27.4 (24.0 ± 2.2)	22.7–23.0 (22.8 ± 0.2)
Snout length	31.5	31.7–38.1 (35.1 ± 2.4)	31.2–35.2 (33.2 ± 2.8)
Percent of Caudal-peduncle length (%)			
Caudal-peduncle depth	94.0	105.9–130.6 (113.1 ± 10.2)	102.5–104.9 (103.7 ± 1.7)
Percent of distance between pectoral-fin origin and pelvic-fin origin			
Pectoral-fin length	73.8	56.9–76.5 (66.9 ± 8.0)	89.0–92.0 (90.5 ± 2.1)
Percent of distance between pelvic-fin origin and anal-fin origin			
Pelvic-fin length	69.1	53.2–66.1 (61.5 ± 5.5)	74.1–76.6 (75.4 ± 1.8)
Dorsal-fin rays	3, 8	3, 8	3, 8
Pectoral-fin rays	1, 10	1, 10–11	1, 10–11
Pelvic-fin rays	1, 6	1, 6	1, 6
Anal-fin rays	2, 5	2, 5	2, 5
Branched caudal-fin rays	16	15–17	16

Body densely scaled, except head and thorax; scales more numerous and larger in males from pectoral to pelvic-fin insertion. Three pairs of barbels, two rostral pairs and one maxillary pair, length of inner rostral barbel one half of outer rostral barbel, reaching anterior nostril; outer rostral barbel reaching posterior nostril, and maxillary barbel reaching posterior margin of eye. Processus dentiformis on upper jaw absent.

Dorsal fin with three unbranched and eight branched rays, origin closer to caudal-fin base than to snout tip, predorsal length 51.3–55.4% SL. Pectoral fin with one unbranched and 10–11 branched rays. Pelvic fin with one unbranched and six branched rays, origin posterior to dorsal fin origin, tips of adpressed fin not reaching anus, longer in males than females (74.1%–76.6% of distance between pelvic-fin insertion and anal-fin origin vs. 53.2%–69.1% in females). Anal fin with two unbranched and five branched rays, origin closer to anus and distant from caudal fin. Caudal fin with two unbranched and 15–17 branched rays (mostly 16). Series of temporal tubercles present on caudal peduncle in nuptial males. Lateral line incomplete, terminating between tip of pectoral fin and dorsal fin origin. 9–11 inner gill rakers on first gill arch. Cephalic lateral system with 12–15+3 infraorbital canal pores, 7–9 supraorbital canal pores, 6–8 supratemporal canal pores, and 6–10 preoperculomandibular canal pores.

Stomach U-shaped (Fig. 2B); intestine long and straight. Swim bladder divided into two chambers; anterior chamber covered by dumbbell-shaped bony capsule, and posterior chamber well developed, connected to anterior chamber by a slender tube, approximately half posterior chamber in length (Fig. 2A).

Coloration. In life, head and trunk with light golden background color. Ventral head and abdomen surface without color pattern. In females, trunk with 15–17 long and twisted dark brown saddles, connected together on dorsal. Some bars bifurcated (Fig. 1H). In males, body with black longitudinal stripe on both sides (Fig. 1D). Fin rays with dark pigments, fin membrane hyaline. In formalin-fixed specimens, lateral stripes and blotches somewhat faded, body generally light yellow.

Sexual dimorphism. Series of temporal tubercles present on caudal peduncle in nuptial male individuals (Fig. 2C), absent in females. Pelvic fin longer in males than females. Males without color patterns except for longitudinal stripes on body sides, females with long and twisted bars on trunks. Tubercles triangular, semi-translucent, angle to the body approximately 30 degrees, yellowish when preserved in formalin.

Distribution and habitat. *Yunnanilus triangulus* sp. nov. is currently only known from Dalongtan Spring, Wangma Village, Huaning County, Yuxi City, Yunnan, China; Nanpan

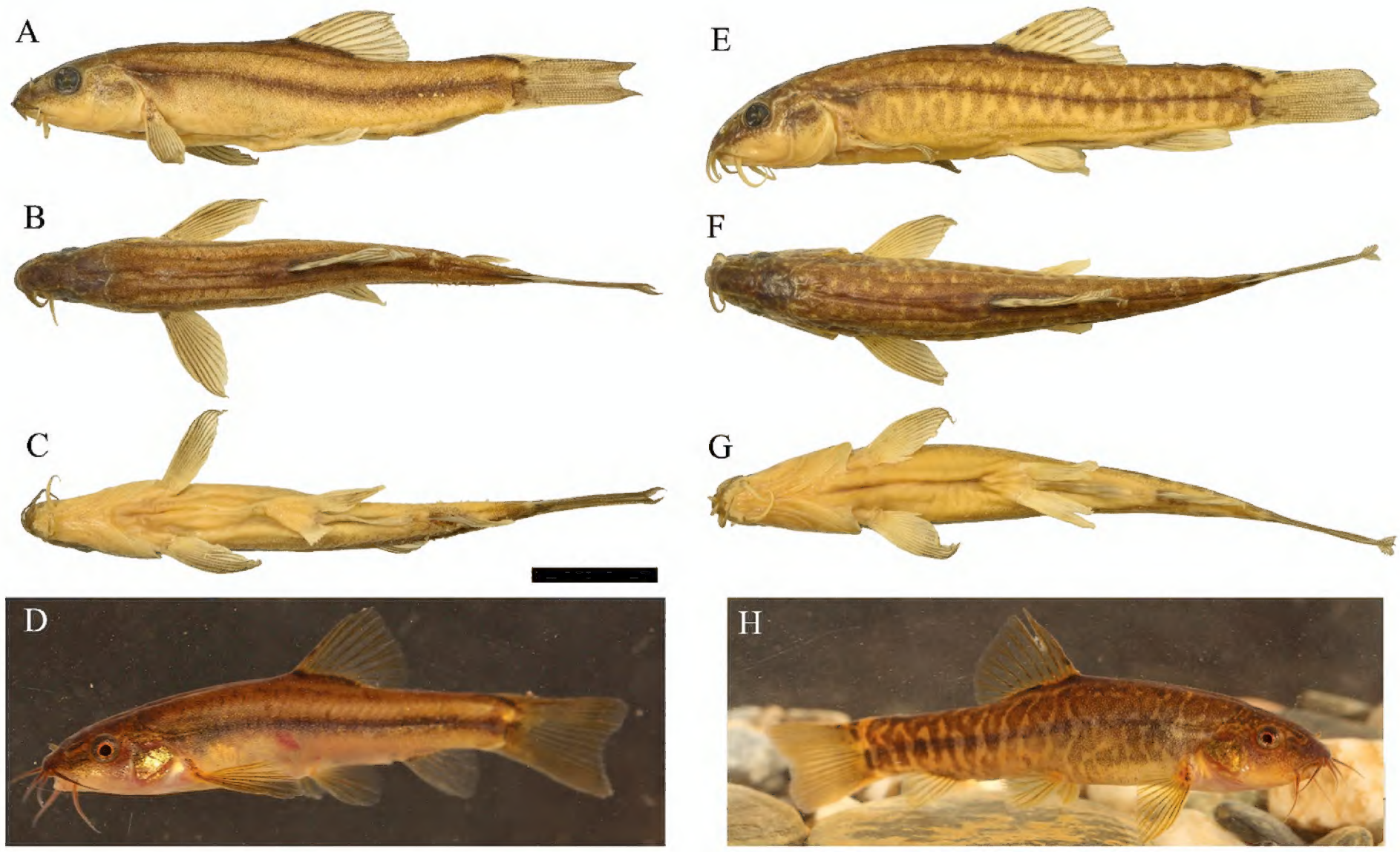

Figure 1. Morphometric characters of *Yunnanilus triangulus* sp. nov. **A–D.** Lateral, dorsal, and ventral views, as well as a living photo of male, paratype KIZ2018002405; **E–H.** Lateral, dorsal, ventral, and living photo of female, holotype KIZ2018002410. Scale bar: 1 cm.

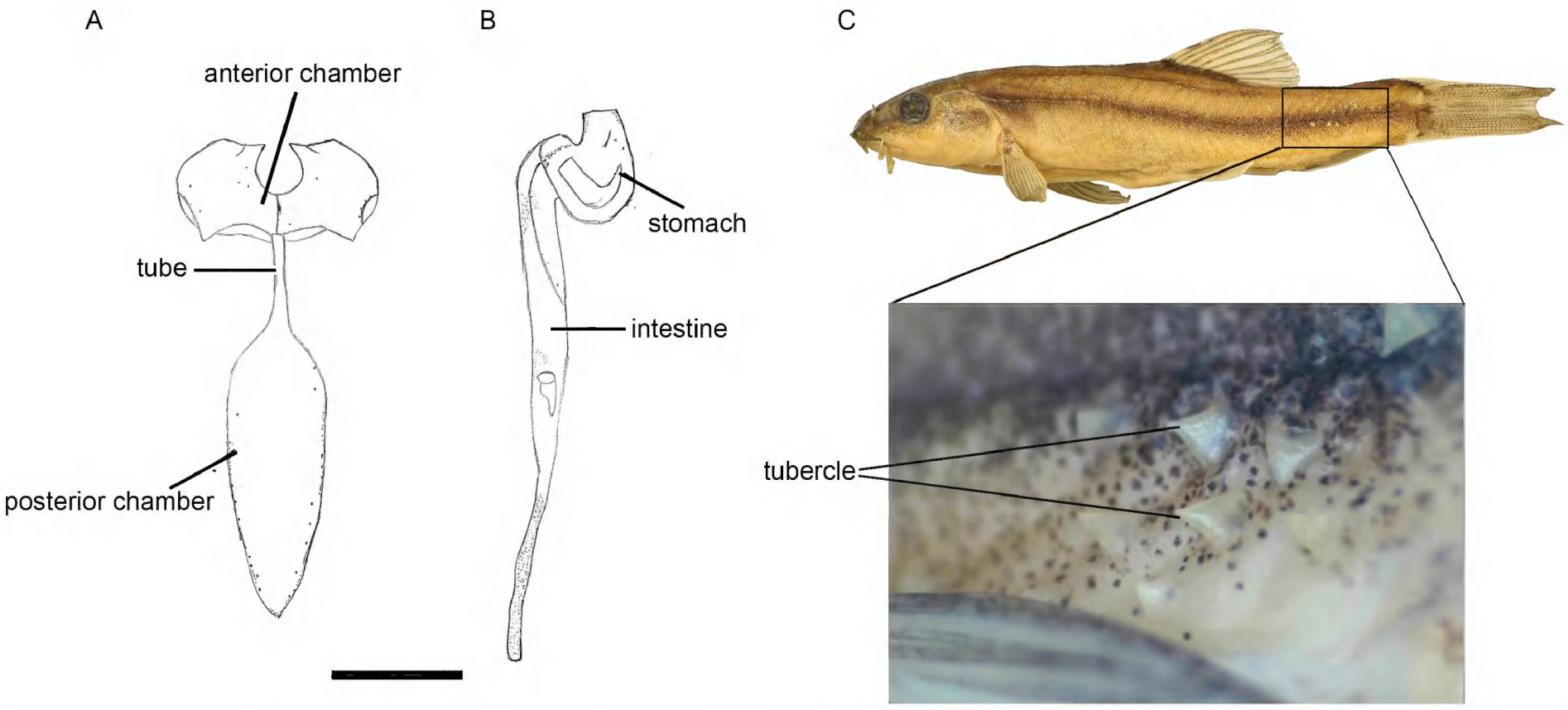

Figure 2. Air bladder (**A**), stomach and intestine (**B**) (KIZ2018002404), and male tubercles on caudal peduncle (**C**) (KIZ2018002405) in *Yunnanilus triangulus* sp. nov.

River (24.1716°N, 102.9182°E, 1619 m a.s.l.). The habitat comprises a deep pool, approximately 3 m in depth and characterized by abundant macrophytes (Fig. 3). Sympatric fish species at the time of collection included *Discogobio yunnanensis*, *Pseudorasbora parva*, and *Carassius auratus*.

Genetic comparisons. In total, 1746 base pairs (670 bp for COI and 1 076 bp for Cyt *b*) from *Yunnanilus triangulus* sp. nov. were amplified and analyzed in this study. These sequences were used for molecular phylogenetic analysis together with 37 complete mitochondrial genomes, 25 Cyt *b* sequences, and 21 COI sequences from GenBank. *Parabotia fasciata* Dabry de Thiersant, 1872, and *Leptobotia elongata* (Bleeker, 1870), two botiid species, served as the outgroups. Given that the BI and ML analyses produced nearly identical topologies, only the BI tree with BPPs and

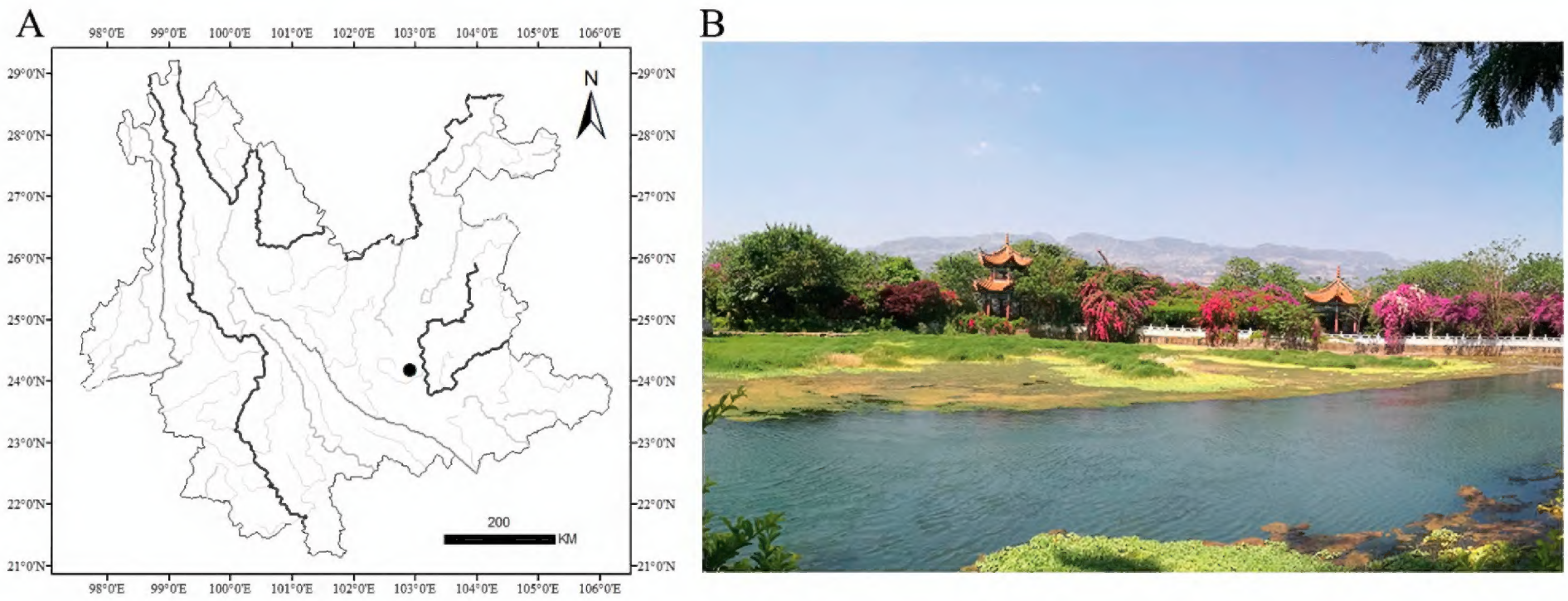

Figure 3. Type locality of *Yunnanilus triangulus* sp. nov. **A.** Distribution map; **B.** Habitat photo of type locality at time of collection.

bootstrap support (BS) values is presented (Fig. 4). The phylogenetic tree strongly supported the placement of *Yunnanilus triangulus* sp. nov. within *Yunnanilus*. The new species also formed a monophyly with *Y. analis*, *Y. chuanheensis*, and *Y. pleurotaenia* (BPP = 1; BS = 99) and exhibited a sister relationship to *Y. jiuchiensis* and *Y. polylepis*. However, the molecular phylogenies did not support the monophyly of *Yunnanilus* itself. In particular, *Yunnanilus yangi* was weakly supported as a sister group to *Eonemachilus* (BPP = 59; BS = 71), forming a clade with other species of *Yunnanilus* (Fig. 4).

The uncorrected *p*-distances of the Cyt *b* and COI genes between *Yunnanilus triangulus* sp. nov. and the other six species ranged from 1.19% to 7.5% (average 3.13%). The maximum and minimum uncorrected *p*-distances were found between *Y. yangi* and *Y. polylepis* and between *Y. analis* and *Y. pleurotaenia*, respectively (Table 2).

Discussion

Molecular analysis unequivocally placed *Yunnanilus triangulus* sp. nov. within the genus *Yunnanilus*. This placement was further corroborated by several morphological features characteristic of the genus, including mouth inferior, lateral and cephalic lateral line pores present, anterior and posterior nostrils separated, an anterior nostril tube-shaped, and a tip not elongated into a barbel-like structure (Du et al. 2023; Qin et al. 2024).

The description of this new species increases the total number of *Yunnanilus* species native to the Yangtze, Pearl, and Red River basins to 21. Notably, *Yunnanilus triangulus* sp. nov. can be distinguished based on a combination of morphological characteristics related to the presence of scales, relative sizes of the eye diameter and interorbital width, absence of a processus dentiformis, lack of an outer gill raker on the first gill arch, and the specific number of branched caudal fin rays. The genus *Yunnanilus* can be broadly divided into two morphological groups based on the presence or absence of body scales. The 'scaleless' group contains *Y. analis*, *Y. beipanjiangensis*, *Y. chui*, *Y. discoloris*, *Y. forkicaudalis*, *Y. paludosus*, and *Y. yangi*, while the 'scaled' group includes the new species together with *Y. chuanheensis*, *Y. elakatis*, *Y. jiuchiensis*, *Y. longibulla*, *Y. macrogaster*, *Y. macrolepis*, *Y. macrositanus*, *Y. nanpanjiangensis*, *Y. parvus*, *Y. pleurotaenia*, *Y. polylepis*, *Y. sichuanensis*, and *Y. spanisbripes*. However, *Yunnanilus triangulus* sp. nov. can be distinguished from *Y. elakatis*, *Y. macrositanus*, *Y. nanpanjiangensis*, and *Y. sichuanensis* by whole body covered by scales, except head and thorax (vs. scales present only on caudal peduncle), from *Y. longibulla* and *Y. jiuchiensis* by eye diameter shorter than interorbital width (vs. longer), from *Y. parvus*, *Y. macrogaster*, *Y. pleurotaenia*, *Y. macrolepis*, *Y. spanisbripes*, and *Y. longibulla* by

Table 2. Uncorrected p-distances (%) between nine species in the genus *Eonemachilus* and *Yunnanilus* based on mitochondrial COI and Cyt *b* genes.

		1	2	3	4	5	6	7	8
1	*Eonemachilus niger*								
2	*E. longidorsalis*	8.14							
3	*Yunnanilus yangi*	9.64	12.08						
4	*Y. polylepis*	9.17	10.64	9.44					
5	*Y. triangulus*	**8.53**	**9.79**	**7.54**	**4.34**				
6	*Y. jiuchiensis*	6.76	7.88	7.29	3.88	**1.89**			
7	*Y. chuanheensis*	9.99	11.33	6.66	5.89	**2.43**	2.77		
8	*Y. pleurotaenia*	8.55	10.20	7.42	4.18	**1.19**	1.86	2.03	
9	*Y. analis*	6.89	7.82	7.46	4.35	**1.37**	2.26	2.32	0.05

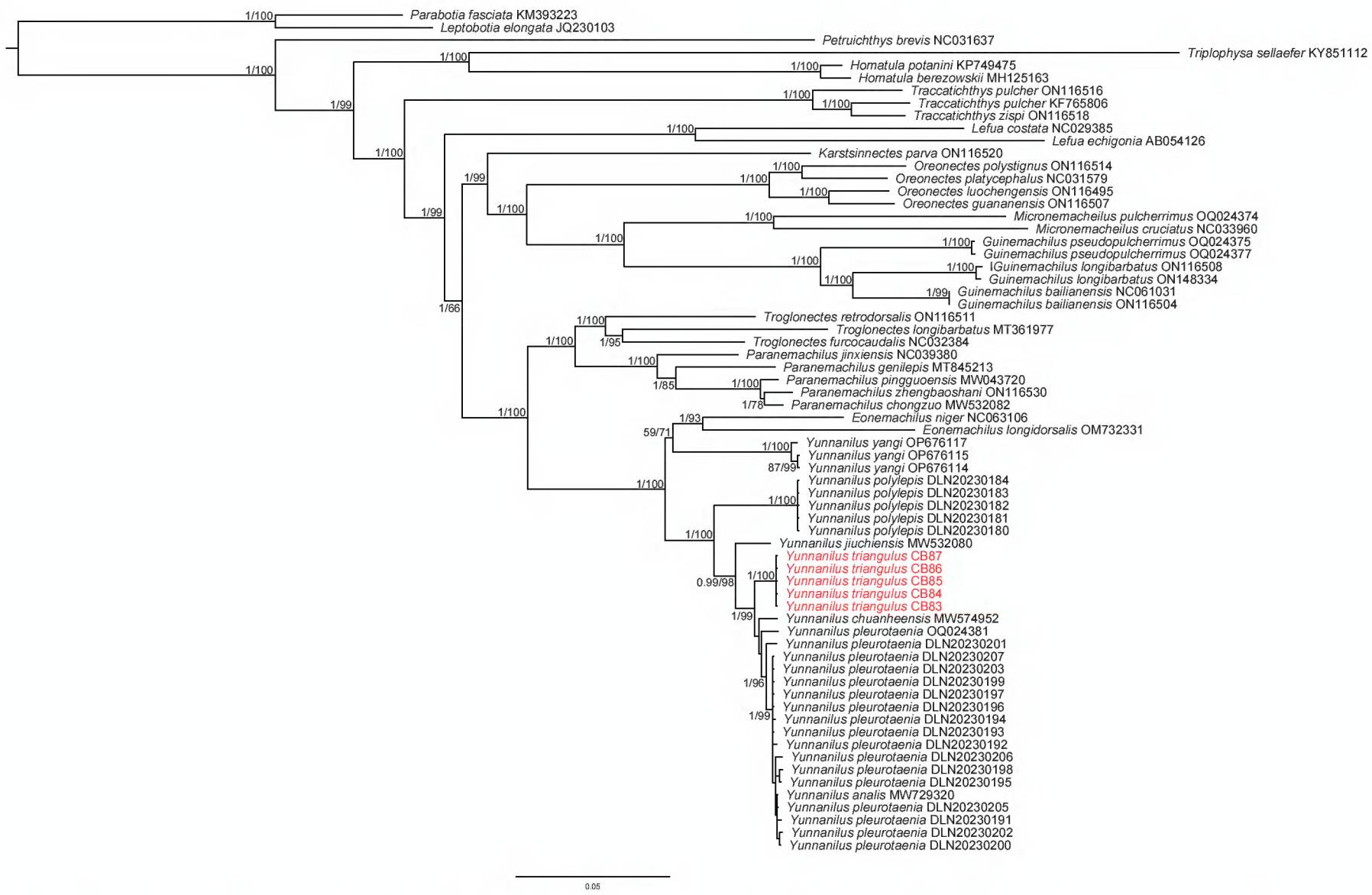

Figure 4. Bayesian phylogram of *Yunnanilus* based on a concatenated dataset of mitochondrial cytochrome c oxidase subunit I (COI) and cytochrome b (Cyt *b*) sequences. Numbers on branches represent BPPs from BI and bootstrap supports from ML.

processus dentiformis absent (vs. present), from *Y. macrolepis* and *Y. spanisbripes* by outer gill raker on first gill arch absent (vs. present), and from *Y. polylepis* by body depth 20.0%–23.0% SL (vs. 15.0%–20.0%) and caudal peduncle depth 94.0%–131.0% of its length (vs. 73.0%–89.0%).

Sexual dimorphism is a common characteristic in members of the family Nemacheilidae. For example, males of the genus *Oreonectes* develop genital papillae posterior to the anus (Du et al. 2008); those of the genera *Triplophysa* and *Barbatula* exhibit thickened and widened outer branched pectoral-fin rays, accompanied by a slender pad of tubercles located anteroventral to the orbit (Zhu 1989). In *Yunnanilus*, sexual dimorphism is often expressed through color pattern variations. Although Qin et al. (2024) documented the presence of small tubercles on the body in nuptial males of *Y. polylepis*, with the presence of triangular tubercles on the caudal peduncle in nuptial males not previously reported. Zhang and Shen (1999) suggested that nuptial tubercles typically occur in areas of the body in contact during courtship or male-male competition, potentially serving to facilitate close physical interaction. Hence, we hypothesize that the caudal peduncle tubercles observed in *Yunnanilus triangulus* sp. nov. play a role in stimulating ovulation in females. However, further studies are necessary to validate this proposition.

Species of *Yunnanilus* are typically confined to small water bodies rich in submerged macrophytes, environments highly vulnerable to anthropogenic impacts and pollution. In September 2024, a survey of the type locality of *Yunnanilus triangulus* sp. nov. revealed that the species had disappeared in the pond, likely due to the rapid decline of submerged macrophytes and the introduction of large numbers of carp into the habitat. The sensitivity of *Yunnanilus* to water pollution, invasive species, and predation has led to consistent population declines across its range. These findings underscore the urgent need for targeted conservation strategies to safeguard *Yunnanilus* species and other freshwater fish in this ecologically significant karst region. It is hoped that this report will stimulate further research and conservation initiatives to address the growing threats faced by freshwater ecosystems in southwestern China.

Nomenclatural acts registration

The electronic version of this article in portable document format represents a published work according to the International Commission on Zoological Nomenclature (ICZN); hence the new name contained in the electronic version is effectively published under the Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclatural acts it contains have been registered in ZooBank LSIDs (Life Science Identifiers) and can be resolved and the associated information can be viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/.

Authors' Contributions

M.L. and L.-N.D. measured the specimens, analyzed the data, conceived and designed the study, and prepared the manuscript, J.-B.L. analyzed the molecular data and constructed the phylogenetic tree. X.-Y.C. provided conceptualization and funding acquisition for the field survey and resources. All authors read and approved the final version of the manuscript.

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